REMARKS

Claims 1-13, 15-18, 20-30, 34, 37-42, 44, 45 and 48-60 are pending in the present application. Claims 1 and 59 are in independent form. In view of the above amendments and the following remarks, favorable reconsideration and allowance of the present application is respectfully requested.

I. 35 U.S.C. § 112, SECOND PARAGRAPH REJECTION

Claim 38 stands rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicants respectfully traverse the rejection.

In particular, the rejection states that "[c]laim 38 requires the fluorescence in step d) of Claim 1 be measured directly on the liquid vehicle without interruption of contact between the liquid vehicle and the contaminants. It is unclear how fluorescence (presumably from soluble enzyme substrates) will be measured on the vehicle rather that in the vehicle. Further, as Claim 1 requires that the liquid vehicle be evacuated through the filter prior to measurement and the filter retains the contaminants, it is unclear how the liquid vehicle can remain in contact with the containments during the measurement step." Action, p. 3.

By the present Amendment, claim 38 is amended to recite "the fluorescence in step d is measured directly in the liquid vehicle without an interruption of the contact between the liquid vehicle and the contaminants."

Thus, the §112, second paragraph rejection to claim 38 is overcome. Accordingly, reconsideration and withdraw of the §112, second paragraph rejection are respectfully requested.

II. EXAMPLE EMBODIMENTS

Example embodiments relate to a series of consecutive steps, which may be summarized as follows: a) concentrating contaminants on the influent side of a filter by passing medium through the filter; b) contacting the influent side of the filter (and thereby the contaminants) with a liquid vehicle containing a substrate such as a methylumbelliferyl derivative; c) allowing substrate and contaminants to interact to produce a detectable moiety (such as a fluorescent compound) in the liquid vehicle; d) evacuating the liquid vehicle through the filter from influent to effluent side; e) detecting the detectable moiety.

Thus, the claimed method includes two separate steps (steps a and d) of passing liquid through the same filter. Steps a and d serve different purposes. Step a serves the purpose of ensuring a high concentration of contaminants on the filter surface in order to facilitate the following steps b and c of interaction with the substrate and production of the detectable moiety. Step d) on the other hand, serves the purpose of interrupting the production of the detectable moiety and isolating it from the contaminants so that step e may subsequently be carried out.

By using the disclosed series of steps, a number of advantages are attained. First of all, compared with conventional methods it is not necessary to manipulate the filter and count microorganisms. Further, the same means (a filter) is used to both obtain a high concentration of the contaminants and to subsequently separate the contaminants from the detectable moiety. In terms of handling a sample, this is clearly superior to any known method in the art because, for example, it is not necessary to employ two different means for obtaining high microorganism counts and for separating microorganisms from detectable moiety. Further, the method also allows a person who is operating "in the field" to take a sample, perform steps a-d on location without access to any laboratory equipment, and then bring a

relatively small volume of filtrate obtained in step d back to a laboratory facility where the determination of the detectable moiety can be performed in step e.

III. CITED ART REJECTIONS

Claims 1-6, 8-13, 15-18, 20, 22-26, 34, 37, 39, 41, 42, 44, 45, 48-52, and 54-60 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Koumura et al. (hereinafter "Koumura"), U.S. Patent No. 4,591,554.

Claims 1-6, 8-13, 15-18, 20-26, 34, 37, 39, 41, 42, 44, 45, 48-52, and 54-60 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Koumura in view of Chen et al. (hereinafter "Chen"), U.S. Patent No. 5,854,011.

Claims 1-13, 15-18, 20, 22-30, 34, 37, 39, 40, 41, 42, 44, 45 and 48-60 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Koumura in view of Tuompo et al. (hereinafter "Tuompo"), U.S. Patent No. 5,714,343.

Applicants respectfully traverse the rejections.

Independent claim 1 is directed to a method for detecting contaminants in a medium suspected of containing such contaminants, the method including the steps of (*inter alia*) "a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device" and "d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter." Applicants submit that Koumura, individually or in combination with Chen and Tuompo, fails to explicitly teach, or otherwise suggest, the above features recited in independent claim 1.

First, the rejection states that "[i]t would have be obvious to one of ordinary skill in the art at the time of the instant invention to modify the method of [Koumura] et al. to use a filter to retain the microbes prior to contact with the

detectable enzyme substrate because the centrifugation step performs an equivalent function to use of a filter." Action, p. 6 (emphasis added).

However, Koumura does not teach, or suggest, a means (e.g., a filter) used for two different purposes. That is, Koumura teaches a step of centrifugation. However, the centrifugation step only serves a purpose similar to that of step d of claim 1, not for the purpose of steps a and d of claim 1.

Furthermore, from column 3, line 54 and column 6, lines 49-50 of Koumura, it is clear that Koumura teaches centrifugation after reaction between microbial cells and methylumbelliferyl derivatives. However, Koumura does not teach, or suggest, that the centrifugation step occurs prior to addition of the methylumbelliferyl derivatives. Thus, Koumura does not teach, or suggest, that the centrifugation step is for the purpose of steps a and d of claim 1.

Even though centrifugation and filtration may serve the same purpose, the solution provided by the claimed invention is much simpler because the microorganisms are already trapped on a filter while they react with the substrate. However, a person exercising the method of Koumura is forced to collect a mixture of medium and cells/cellular debris, to perform centrifugation of the mixture, to separate medium from cells/debris which has been spun down, and then to perform a measurement on the medium. The method of claim 1 merely requires that the liquid vehicle is forced through the filter, and then the measurement may then be performed directly on the liquid vehicle. After the production of the fluorescent moiety, Koumura's method includes several steps that include handling of the sample, where only the filtration step is necessary in the method recited in claim 1.

In addition, Koumura's method does not entail the other above-discussed advantages relating to field use of the claimed method. If a field operator, for example, were to use Koumura's method to test an environmental sample "in the

field," the sample either would have to be brought back to a laboratory to carry out the cultivation and centrifugation steps, or, alternatively, the field operator would have to bring equipment for culturing and centrifugation. Koumura does not teach, or suggest, that a simpler or more convenient solution would exist.

Koumura uses a centrifugation step after contact with the enzyme substrate. Koumura does not teach, or suggest, that the same means, let alone the same filter, is used in order to obtain sufficient concentration of microorganisms and to later separate microorganisms from substrate. Thus, Applicants submit that one would not be motivated to use the filter to retain the microbes prior to contact with the detectable enzyme substrate (as alleged by the Examiner), absent inappropriate hindsight of the Applicants' own disclosure.

Secondly, Koumura's technology relies on a cultivation step wherein microorganisms are propagated in a nutrient containing medium which also contains the substrate. No such step is necessary according to example embodiments of the present application.

In particular, the rejection states that "[i]t is inherent in the method of Koumura *et al.* that the use of peptone as a non-selective growth-enhancer will increase the overall sensitivity in the detection step as the growth and multiplication of microbes will increase the amount of endogenous enzymes able to interact with the detectable substrate and increase the released signal (detectable moiety)." Action, p. 5.

The Examiner's assertions emphasize the fact that Koumura utilizes a nutrient medium in order to propagate microorganisms prior to the detection. However, researchers in microbiology have long known that cultivation/multiplication of an environmental sample of bacteria will result in a bacterial population which is distinctly different from the original bacterial population, as different bacteria species grow with very different growth rates on

different growth media and under different growth conditions (e.g., aerobic bacteria will benefit from ample oxygen, whereas anaerobic bacteria will not). The enzyme activity after the multiplication step will therefore not reflect the original endogenous bacteria population, rather it will represent the activity of rapidly growing opportunistic bacteria at the expense of slower growing bacteria or species with special growth requirements. This makes it impossible to extrapolate the result following a multiplication step to an estimate of the initial population size. The exclusion of a culturing step is therefore an advantage in the present application because the signal obtained in the final measurement will more accurately reflect the concentration of microorganisms in the environmental sample tested.

Applicants summarize the differences between Koumura and example embodiments below:

Feature	**Claimed invention**	**Koumura**
Concentration of microorganisms on filter	+	- (cultivation is used to propagate microorganisms)
Interrupting production of detectable moiety by filtration using same filter as above	+	- (centrifugation is used)

Thus, Koumura fails to teach, or suggest, both of steps a and d of claim 1. More specifically, Koumura fails to teach, or suggest, the use of the same means for carrying out steps a and d of claim 1.

Thirdly, in both Koumura et al. and in the present application, enzyme activity correlates with the number of bacteria in a sample. Measurable enzyme activity in a given sample is influenced by many factors, but the two most

important are 1) enzyme concentration and 2) substrate concentration. Each of these two variables is discussed below in relation to the two methods.

Enzyme concentration

The enzyme activity is directly proportional to the concentration of enzyme. This means that increasing the enzyme concentration (*i.e.* the concentration of bacteria) 10 times also increases the concentration of fluorophore formed by the enzyme reaction by a factor 10.

In the method disclosed in the present application, bacteria may, for example, be concentrated from a water sample of 1,000 ml to a 0.5 ml volume (which is the dead volume in the filter housing currently used by the inventors) prior to the enzyme reaction (*i.e.* providing a concentration of bacteria with a factor 2,000). After that, the filter housing is saturated with an enzyme substrate solution equal to the dead volume of 0.5 ml.

The method of Koumura teaches that a sample of is contacted with an enzyme substrate and incubated in order to increase the number of bacteria and thereby increase the enzyme concentration. Typically, Koumura uses total volumes of 11 ml. See Examples 2, 3, 5, 6, 7, 8 of Koumura. So, the enzyme concentration, which may be attained according to Koumura, is the enzyme concentration of the mixture between the nutrient medium and the bacterial sample, and a centrifugation step prior to detection in Koumura does not change these facts.

In contrast, the method disclosed in the present application teaches a method which in one rapid step can drastically increase the enzyme concentration (e.g. 2,000 times) and thereby sensitivity, where Koumura has to increase the number of microorganisms by culture to attain a concentration that allows detection. In column 4, lines 6-9, Koumura mentions that microorganism may be counted directly and, for example, be concentrated (no means for concentration are

mentioned) prior to measurement. However, in the following paragraph, Koumura states that this approach is held not to work for bacterial numbers lower than 10^3/ml. So, as soon as the concentration of microorganisms in Koumura is unknown or very low, a culturing step has to be included.

Substrate concentration

Enzyme reaction rate varies with substrate concentration. Increasing enzyme substrate concentration increases enzyme reaction rate and vice versa. The present application, for example, has in practical use enabling the assaying of 1000 ml sample by using 0.5 ml substrate solution (present in the dead volume in the filter housing) containing only 32 µg MUP substrate.

In the teachings of Komura, the assay of 1000 ml liquid sample with a content of 32 µg MUP would result in a dramatic decrease in substrate concentration and a reduction of enzyme reaction rates close to zero. And, it would also have the consequence that much higher amounts of substrate should be used if this dramatic decrease was to be avoided. In order to achieve a reaction rate in Koumura that is similar to the reaction rate found in the present application, a 2000 times higher amount of MUP substrate will have to be added to the assay liquid (simply because the volume is so much larger). In other words, the substrate consumption in an assay of 1000 ml liquid sample is in this case 2000 times higher in the teachings of Koumura than in the present application.

It can be noted that that the amount of substrate used (e.g., in Example 2) in Koumura is approximately 2770 µg (1.1×10^{-2} M MUP in 1.0 ml buffer, where the molecular weight of 4-MUP is about 252 – this provides for 0.002772 g MUP, *i.e.* 2772 µg), and given the fact that the MUP substrate is relatively expensive, the present invention also provides for a marked cost reduction.

Both these examples demonstrate that the concentration of microorganism via retention on a filter can render the presently claimed method extremely sensitive by simple and non-time consuming means. In contrast, Koumura indicates that it is necessary to culture microorganisms in order to obtain sufficient numbers of microorganisms in the detection phase. That is, the method disclosed in Koumura by necessity is more time consuming than simply concentrating microorganisms on a filter as recited in the claimed method.

Fourthly, Tuompo is directed to a method for the detection of microorganisms. The filter used in Tuompo is not used for both "a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device" and "d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter" as recited in independent claim 1.

Chen, which is directed to a rapid method for detecting yeasts and/or molds in a sample, fails to even teach, or suggest, the use of a filter. Thus, Chen fails to teach, or suggest, "a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device" and "d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter" as recited in independent claim 1.

Thus, Chen and Tuompo fail to cure the above deficiencies of Koumura with respect to independent claim 1.

For at least these reasons, Applicants submit that Koumura, individually or in combination with Chen and Tuompo, fails to explicitly teach, or otherwise suggest, a method for detecting contaminants in a medium suspected of containing such contaminants, the method including the steps of "a) passing a known volume of said

medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device" and "d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter" as recited in independent claim 1.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the §103(a) rejections to independent claim 1, and claims 2-13, 15-18, 20-30, 34, 37-42, 44, 45 and 48-58 at least by virtue of their dependency on independent claim 1.

Independent claim 59 is directed to a method for detecting contaminants in a medium suspected of containing such contaminants, the method including the steps of (*inter alia*) "a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device" and "d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter." Thus, independent claim 59 is patentable over the combination of Koumura, Chen and Tuompo for similar reasons as given above with respect to independent claim 1.

Thus, Applicants respectfully request that the Examiner reconsider and withdraw the §103(a) rejections to independent claim 59, and claim 60 at least by virtue of its dependency on independent claim 59.

CONCLUSION

Accordingly, in view of the above, reconsideration of the objection and rejections and allowance of each of claims 1-13, 15-18, 20-30, 34, 37-42, 44, 45 and 48-60 in connection with the present application is earnestly solicited.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicants hereby petition for a one (1) month extension of time for filing a reply to the outstanding Office Action and submit the required $65.00 extension fee for a small entity herewith.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 08-0750 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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